Proc. 7th Int Wheat Genet Symp
Miller TE and Koebner RMD, eds,
IPSR, Cambridge, pp 455–460 (1988)

Chromosome breakage in wheat induced by the gametocidal gene of *Aegilops triuncialis* L.: Its utilization for wheat genetics and breeding

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Since the common wheat cultivar Norin 26 (N26) has the suppressor for the gametocidal gene on chromosome 3C of *Ae. triuncialis*, the F1 between N26 and the disomic addition line of cultivar Chinese Spring, possessing a pair of chromosome 3C, (CS+3C3C) is fully fertile. In the progeny of the F1, however, a high rate of chromosome breakage occurs. On the other hand, no breakage occurs in the progeny of the F1 between N26 and normal Chinese Spring (CS) or between CS and CS+3C3C. Thus, it is obvious that the chromosome breakage results from an interaction between a gene(s) from N26 and a gene(s) from chromosome 3C. Chromosome breakage was observed only in the selfed progenies of the monosomic addition, but not in the substitution lines, which were produced by the cross between CS+3C3C and mono-3B of Shin-chunaga, a parent cultivar of N26. The location of the gene(s) responsibile for the breakage was located on chromosome 3B. Additionally, by observing mutation in the selfed progenies of F1s between CS+3C3C and a hybrid between N26 and CS, the gene from N26 which causes breakage was found to be *Igc1* or a closely linked gene. Some progenies that were derived from plants that did not carry the *Igc1* gene also had chromosome mutations, but at a lower frequency, suggesting that a minor gene(s) of N26 other than *Igc1* is concerned with chromosome breakage. From the results we discuss the relationship between chromosome breakage and gamete abortion, both of which are possibly caused by the same genes. The chromosome breakage indicated here is useful for producing chromosome deletion lines that are applicable in wheat genetics and transfer of alien genes to common wheat.

Aegilops triuncialis carries a gametocidal gene, *Gc-C*, on chromosome 3C. Monosomic additions of chromosome 3C in a Chinese Spring background have reduced fertility, because gametes lacking the 3C chromosome and hence the *Gc-C* gene are killed. Consequently, the monosomic addition line is semisterile, and chromosome 3C transmits preferentially to the progeny (Endo & Tsunewaki 1975). On the other hand, when chromosome 3C is added to the cultivar Norin 26, it does not act as a gametocidal chromosome (Endo 1978). Tsujimoto and Tsunewaki (1985a) indicated that Norin 26 has a suppressor of the gametocidal gene, *Igc1*, on chromosome 3B. Chromosome 3C in the monosomic addition line produced by the cross between Norin 26 and the disomic addition line of Chinese Spring carrying chromosome 3C, CS+3C3C, behaves like an ordinary alien chromosome added to common wheat. The F1 monosomic addition is fully fertile and also cytologically normal, i.e., 2n=42+3C and 21"+1' in mitotic and meiotic metaphase, respectively. In the progeny, however, many plants show chromosome breakage. The chromosomes are broked by the interaction between a gene(s) from Norin 26 and a gene(s)

Table 1. Selfed seed fertility of monosomic addition and substitution lines produced by the cross between Shin-chunaga mono-3B and disomic addition line of Chinese Spring carrying chromosome 3C (CS+3C3C), and the chromosome breakage in their selfed progenies.

Line	F_1 plant no.	Selfed seed fert.(%)	No. of F_2 plants observed	Chromosome breakage (β)
Addition	1	90	10	80.0
	2	78	9	22.2
	3	88	5	60.0
	Total	85	24	54.2
Substitution	1	15	7	0.0
	2	12	10	0.0
	3	12	9	0.0
	Total	13	26	0.0

from chromosome 3C of *Ae. triuncialis* (Tsujimoto & Tsunewaki 1985a,b).

The aim of the present study was to clarify the relationship of the gene(s) and genetic mechanism between the abortion of gametes and chromosome breakage, and to discuss the utilization of the chromosome breakage in wheat genetics and breeding.

MATERIALS AND METHODS

Plant materials:

The lines used in the present experiments are the disomic addition line of Chinese Spring possessing a pair of chromosome 3Cs (CS+3C3C), monosomic 3B of cultivar Shin-chunaga and euploid of Chinese Spring (CS), Norin 26 (N26), and Shin-chunaga (Scn). Shin-chunaga is a parent of Norin 26 and a carrier of *Igc1*.

Chromosome observation and evaluation of chromosome breakage:

The usual acetocarmine squash method was applied to observe chromosomes in root tip meristem cells. Abnormal chromosomes were recorded. We suppose that telo- or acrosomes are produced by a single breakage event, whereas dicentric or ring chromosomes arise by two events. Based on this supposition, we calculated the index of chromosome breakage (β) by the following formula:

$$\beta = 100 \sum_{i=1}^{n} (t_i+2d_i)/n$$

where t, d, and n are the number of telo- or acrosomes in the karyotype of a plant, the number of dicentric or ring chromosomes, and the number of plants in the line, respectively. When the chromosome constitution is chimeric among the cells, which occasionally happens, that constitution having the least chromosome breakage is regarded as the representative.

Judgment of mutants:

In the present wheat lines, speltoid is the only character which is detected easily and explained genetically as lack of the *Q* gene on chromosome arm 5AL. However, we observed abnormal plants although no hybrid weakness was expected in the progeny of the hybrids between CS, N26, and Scn, and chromosome 3C which does not have a gene(s) responsible for hybrid weakness. Consequently, the plants showing abnormal phenotypes were regarded as mutants which were possibly produced as a result of chromosome breakage and consequent deletion of the responsible gene(s), although their genetic bases were not known. Such mutants, which appeared to resemble a nullisomic or ditelsomic plant, were clearly distinguished from weak plants caused by unfavourable environmental conditions because they had uniform morphology in each spike,

Table 2. Chromosome breakage and speltoid mutants in the progenies of monosomic addition lines produced by the cross between the disomic addition line of Chinese Spring carrying chromosome 3C (CS+3C3C) and Chinese Spring (CS) or Norin 26 (N26).

Cross combination	Chromosome breakage		Speltoid mutant	
	No. of plants observed	β	No. of plants observed	%
(N26 x CS+3C3C) x CS	61	62.2	104	12.5
CS x (N26 x CS+3C3C)	47	8.5	58	3.5
(CS x CS+3C3C) x CS	32	0.0	32	0.0
CS x (CS x CS+3C3C)	19	0.0	20	0.0
(N26 x CS)F_2	20	0.0	20	0.0

leaf and culm.

Seed fertility:
Seed fertility was evaluated as the percent of seed set in the first and second florets of well-developed spikelets.

RESULTS

Chromosome location of a gene(s) in common wheat for chromosome breakage

Since monosomics of N26 are not available, monosomic 3B of Scn was used in this experiment. Scn mono-3B was crossed as female to CS+3C3C, and three monosomic addition plants (2n=42+3C) and three monosomic substitution plants (2n=42-3B+3C) were selected. All the monosomic addition plants were fully fertile because they had *Igc1* on chromosome 3B derived from Scn. On the other hand, all the monosomic substitution plants were semi-sterile because of the lack of Scn's 3B chromosome (Table 1). Both the lines were self-pollinated and chromosomes in the F_2s were observed (Table 1). Chromosome breakage was observed only in the F_2s derived from the monosomic addition line. This result clearly indicates that the gene(s) responsible for chromosome breakage is located on chromosome 3B of Scn and probably on that of N26, on which *Igc1* is also located.

Linkage between *Igc1* and a gene(s) responsible for chromosome breakage in N26

To determine the linkage between *Igc1* and the gene(s) responsible for chromosome breakage, the F_1 between N26 and CS was crossed as pollen parent to CS+3C3C, and a monosomic addition line was produced. In this line plants segregated in terms of fertility, i.e., fertile and semi-sterile plants having or lacking *Igc1*, respectively. Twenty-nine fertile and 31 semi-sterile plants were self-pollinated, and mutants, instead of chromosome breakage, were looked for among the progenies. Many chromosome breaks cause phenotypic abnormality. In fact, we observed mutants only in the lines having chromosome breakage (Table 2). Thus, we assumed in this experiment that the percentage of mutants reflects the chromosome breakage rate.

Figure 1 shows the percentage of mutants among the progenies of both fertile and semi-sterile plants. Although the percentage of mutants in the two groups overlap one another, plants in the two groups were clustered differently. If recombination between *Igc1* and the gene(s) responsible for chromosome breakage occurred, both the groups in Figure 1 might have two clear peaks and overlap in one of them. Consequently, the result obtained here indicates that no recombination occurred

between *Igc1* and the gene for chromosome breakage. Thus, the gene of N26 for chromosome breakage is *Igc1* itself or a gene tightly linked with it. At present it is reasonable to conclude that *Igc1* has a pleiotropic effect causing chromosome breakage in the presence of chromosome 3C of *Ae. triuncialis*.

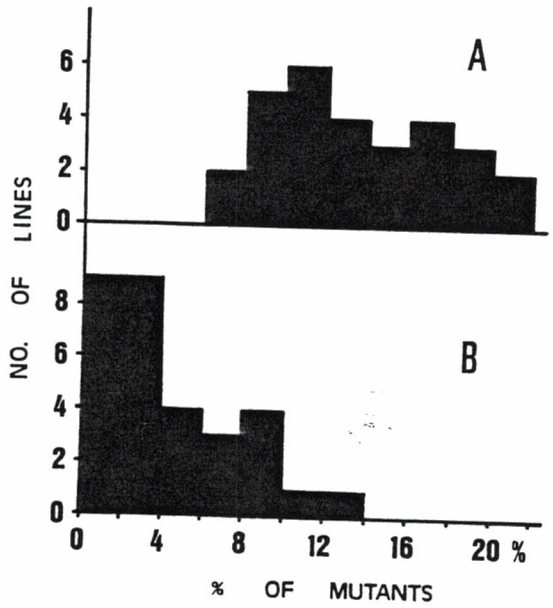
DISCUSSION

Genes and mechanism of chromosome breakage

The present studies suggest that *Igc1* of N26, the suppressor of the gametocidal effect of *Gc-C*, has another function, that of inducing chromosome breakage in cooperation with a gene on chromosome 3C. This may indicate the possibility that the gene on chromosome 3C for chromosome breakage is also the gametocidal gene, *Gc-C*, although linkage analysis of the genes on chromosome 3C is not possible. If so, gamete abortion or semi-sterility oberved in CS+3C may be attributable to severe chromosome fragmentation. But it is the fertile monosomic addition line that causes chromosome breakage in the progeny. If *Igc1* has a function to suppress the severe chromosome fragmentation caused by *Gc-C*, it acts as a suppressor of gamete abortion. However, if this suppressor action of *Igc1* is not complete, *Igc1* would look like an enhancer of chromosome breakage because gametes carrying only a few breaks do not abort and are transmitted to the next generation. If this explains the nature of the phenomena correctly, only gametes lacking chromosome 3C should have chromosome breakage. But in the lines produced by the cross, (CS+3C3C x N26)F_1 x CS and its reciprocal, plants carrying chromosome 3C also had chromosome breakage (Tsujimoto & Tsunewaki 1985a). More detailed analyses are necessary to explain the nature of these phenomena.

In the progenies of the semi-sterile plants we observed up to 13.8% mutants. This suggests that a gene(s) on chromosome 3C has a potential function to induce breakage and that a minor factor(s) of N26 other than *Igc1* is involved in the chromosome breakage.

Figure 1. Percentage of mutation in the selfed progenies of the fertile (A) and semi-sterile (B) plants which were obtained by the cross, CS+3C3C x (CS x N26).

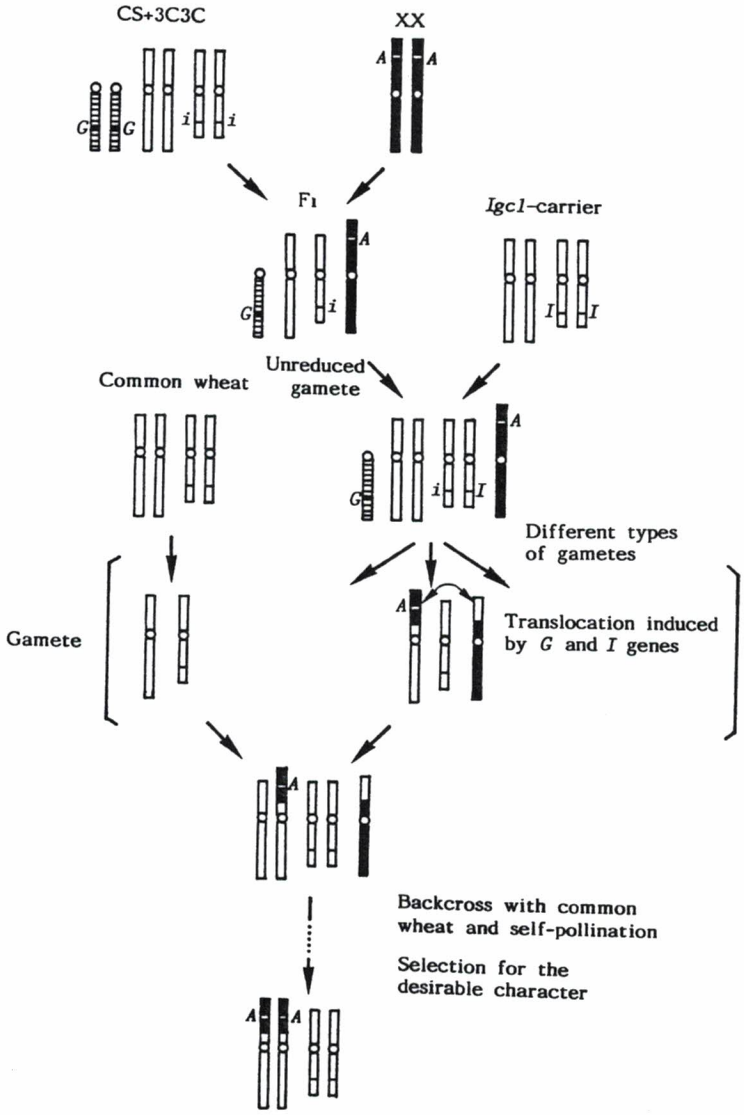


Tsujimoto and Tsunewaki (1988) observed that *Gc1a* and *Gc1b* originating from *Ae. speltoides* induced chromosome breakage only in aneuploids, and supposed that the genomic imbalance enhanced it. Likewise in the present case, the genomic imbalance caused by the inter-varietal cross may enhance chromosome breakage.

Utilisation of chromosome breakage for wheat genetics and breeding

We have already produced several chromosome deletion lines by use of the present genes or the other gametocidal genes, *Gc1a* and *Gc1b*, on chromosome 2B of common wheat derived from *Ae. speltoides* (Tsujimoto 1987). These lines are useful for mapping genes more

Figure 2. Method to transfer a gene (*A*) of alien genome (X) to a common wheat chromosome with *Igc1* gene (*I*) and the gametocidal gene of chromosome 3C (*G*).



precisely. Additionally, the chromosome rearrangement may be used to transfer an alien gene to common wheat. For this purpose, the present genes may be more useful than *Gc1a*, *Gc1b* or the other genes of *Ae. longissima* or *Ae. sharonensis* because stable plants, which lack the gametocidal chromosome, 3C, and produce no more breakage, may be produced by *Igc1*, i.e., the suppressor of the gametocidal gene. On the other hand, in the case of the other genes, it is difficult to eliminate the gametocidal chromosomes. A method for alien gene transfer using the present genes has already been proposed (Tsujimoto 1986, Tsujimoto & Noda 1986). The method is briefly illustrated in Figure 2. CS+3C3C is crossed to a related species of wheat having a desirable gene, *A*, in its genome, X, then the F_1 is crossed to a common wheat carrying *Igc1*. Some of the gametes produced should have a wheat chromosome to which an alien chromosome segment carrying the desirable gene has been translocated by chromosome breakage and fusion. The F_1 hybrids are then backcrossed or self-pollinated and their progenies are selected for the desired character, chromosome pairing, and normal fertility. If an alien addition line of common wheat carrying the desirable gene is available for the first cross, the following steps will be easier.

Acknowledgements

We are grateful to Dr T. Nagayoshi, Faculty of Agriculture, Kobe University, Japan, who kindly provided the monosomic 3B of Shin-chunaga. This work was partly supported by a grant-in-aid (No. 62740366) from the Ministry of Education, Science and Culture, Japan, and one from Nippon Life Insurance Foundation. Contribution (No. 48) from the KiharaInstitute for Biological Research, Yokohama City University.

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